DISCOVERY

To Cite:
Abdel-Salam OME, El-Shebiney SA. The first application of Direct Analysis in Real Time (DART+) with the use of NIST-14 for the detection of delta-9-tetrahydrocannabinol in cannabis seizures in Egypt. *Discovery* 2023; 59: e74d1267

Author Affiliation:
Department of Toxicology and Narcotics, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt

***Corresponding author**
Department of Toxicology and Narcotics, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt
Email: omasalam@hotmail.com

Peer-Review History
Received: 21 March 2023
Reviewed & Revised: 25/March/2023 to 09/May/2023
Accepted: 13 May 2023
Published: June 2023

Peer-Review Model
External peer-review was done through double-blind method.

Discovery
pISSN 2278–5469; eISSN 2278–5450

The first application of Direct Analysis in Real Time (DART+) with the use of NIST-14 for the detection of delta-9-tetrahydrocannabinol in cannabis seizures in Egypt

Omar ME Abdel-Salam*, Shaimaa A El Shebiney

ABSTRACT

The aim of this study was to detect the presence of delta-9-tetrahydrocannabinol (THC) in cannabis seizures in Egypt using the new mass analysis tool "direct analysis in real time" (DART+). The dried flowering tops and leaves (marijuana), the cannabis resin (hashish) or the dried stalks were subjected to analysis using direct analysis in real time (DART+). Results indicated that the use of DART+ for leaves, resin and even dried stalks without extraction displayed a single peak for THC. It is concluded that THC was the major compound identified. The use of the new mass analysis tool DART+ proved to be a simple and rapid technique for detecting the presence of THC in seized samples.

Keywords: Cannabis, hashish, marijuana, direct analysis in real time, DART+

1. INTRODUCTION

Cannabis sativa L. (family *Cannabidaceae*) remains the most common illicit drug world-wide; with an estimated 192 million people having using the substance at least once in the year 2018 and this number is on the rise. Cannabis is cultivated in 151 countries in the period 2010–2018 and globally, 4.303 and 1.307 tons of cannabis herb and resin, respectively, were seized in 2018 (World Drug Report, 2020). Cannabis is mainly abused for its mood-altering effects, producing mild euphoria and relaxation. There are also intensification of sensory experiences and distorted perception of time (Huestis, 2002). The use of cannabis is common to all age groups, but adolescents, being in a critical period for brain maturation are the most susceptible to its effects with a resultant change in emotional behavior and an increase in the likelihood for seeking and trying other addictive substances (Dahl, 2004; Abdel-Salam, 2019).

Cannabis in the form of the flowering tops and leaves (marijuana) or the compressed resin (hashish) contains delta-9-tetrahydrocannabinol (THC) as the principal pharmacological ingredient which is responsible for the psychoactive

DISCOVERY SCIENTIFIC SOCIETY

properties of the plant (Mechoulam and Gaoni, 1967). Cannabis also contains 120 other cannabinoids including cannabidiol, cannabinol, tetrahydrocannabivarin, cannabigerol, cannabidivarin and cannabichromene. These are present however, in much lower concentrations than the main cannabinoid THC (El-Shohly, 2002).

Despite changes in recreational cannabis regulation in the past years, with legalization of the non-medical use of cannabis in a number of US states and other countries, the possession and/or use of cannabis is still prohibited in many countries all over the world including Egypt (Abdel-Salam et al., 2017; World Drug Report, 2018). Hence, the detection of the major psychoactive constituent THC in seized samples of cannabis is an important step in forensic laboratories for its identification and therefore criminalization.

The analytical techniques in use for the detection of THC and its metabolites in seized samples include high performance liquid chromatography (HPLC), liquid chromatography (LC) coupled with mass spectrometry (MS) and gas chromatography/mass spectrometry (GC/MS). The latter equipment is the most commonly used one in drug analysis (Stolker et al., 2004; Gambaro et al., 2002; Ilias et al., 2005). These techniques, however, are time consuming and require extraction/derivatization of the sample.

The direct analysis in real time (DART) coupled with time-of-flight mass spectrometry provides the molecular information for different compounds in samples. The technique has been reported to be a simple and an efficient method for "ambient ionization" for the screening of the targeted compounds in the sample (Kawamura et al., 2011; Chernetsova et al., 2011). The technique has been used for the rapid detection of synthetic cannabinoid analogues in herbal samples (Musah et al., 2012) and designer drugs (Brown et al., 2016). The aim of this study was to detect the presence of THC in seized samples of cannabis in Egypt using the new analysis tool direct analysis in real time mass spectrometry (DART+).

2. MATERIAL AND METHODS

Cannabis seizures

Cannabis seizures were officially obtained from the Ministry of Justice, Egypt. The seizures were marijuana i.e., the flowering tops and leaves of the plant *Cannabis sativa* L. and hashish which is the compressed resin. The seizures were subjected to morphological examination with the use of light microscopy and preliminary color test. Macroscopically, Marijuana samples were dark green loose crushed leaves and flowering tops with some seeds present. Hashish samples were dark brown compressed cubes of various sizes. The two types had characteristic fragrance.

Analysis of cannabis constituents

Direct analysis in real time (DART+)

The JEOL DART+ Accu TOF mass spectrometer (JMS-T100LC; Jeol, Japan) was used. The needle voltage was set to 3500 V, heating element to 300 ºC and he gas flow to 4 L/min. For the mass spectrometer, the ion source temperature was set to 300 °C, with helium as the ionizing gas. The TOF-MS was set with a peak voltage of 1500 V, a reflection voltage of 900 V and a pusher bias voltage of -0.52 V and a detector voltage of 2500 V. Each sample was slowly moved into the ion stream about halfway between the ion source and the analyzer orifice, while looking for the response in the real time chronogram. The sample to be analysed was rapped in clean tissue paper and put between the ion source under vacuum and the gas gun. A clean tissue paper was run then the plant material was rapped in this tissue paper and another run was done. The last run was then subtracted from the clean tissue paper run. The dried stalks of the *Cannabis sativa* were applied directly between the ion source under vacuum and the gas gun.

The obtained spectra were background corrected. Molecular formulae were identified using elemental composition and isotope matching programs in the Jeol Mass Center Main Suite software (JEOL USA, Peabody, MA). The suggested molecular formulae were assigned with a confidence level greater than 90%. Identification of the resultant molecular weights to chemical compounds was performed by using the library NIST 14 using Mass mountaineer.

3. RESULTS

Direct analysis in real time (DART+)

The mass analysis tool DART+ was used for analyzing leaves and resin without extraction. The leaves displayed a high peak for THC at 315.23 (abundance 100) and a minor peak for HU-336 at 329.21. Some minor cannabinoids; cannabinol at 311.19 (abundance 34.18), cannabidiol at 315.23 (abundance 100) were identified by the DART library NIST14. Surprisingly, synthetic cannabinoids were also detected; HU-345 at 325.18 (abundance 5.47), JWH-175 at 328.20 (abundance 6.84) (Figure 1). Figure 2 shows a high peak for THC at 315.23 (abundance 100) and a minor peak for HU-336 at 329.21 (abundance 9.411). Other cannabinoids identified by the library were cannabidiol at 315.23 (abundance 100) and HU-331 at 329.21(abundance 9.411).

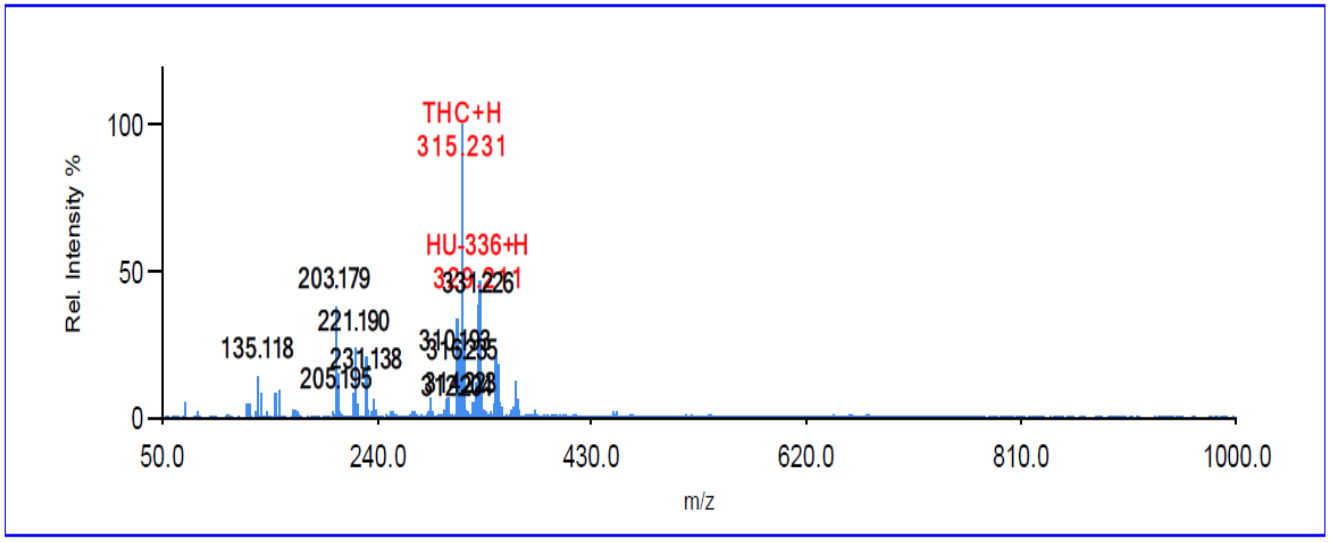

Figure 1 DART+ chart showing the unique peak of THC ([M+H]⁺) at M+ 315.231 in *Cannabis sativa* leaves

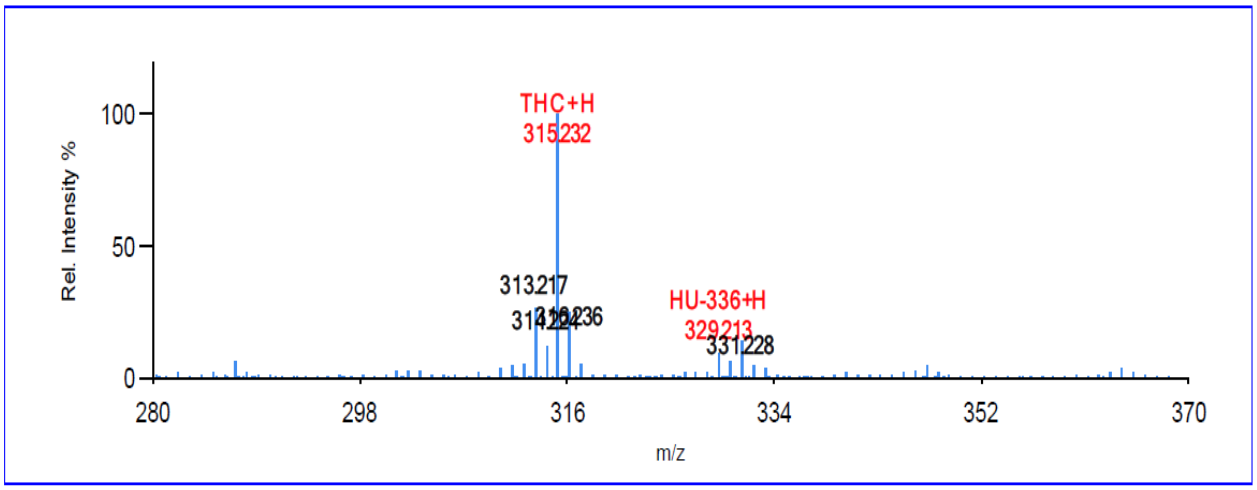

Figure 2 DART+ chart showing the presence of the presence of THC peak at M+ 315.231 ([M+H]⁺) in *Cannabis sativa* leaves

On the other hand, the resin sample showed a high peak for THC at 315.23 (abundance 100) and a minor peak for cannabinol at 311.2 (abundance 12.356). Other cannabinoids detected by the library were cannabidiol at 315.232 (abundance 100), HU-336 and HU-331 at 329.21 (abundance 11.578 and 11.578, respectively) (Figure 3). Figure 4 shows a high peak for THC at 315.23 (abundance 100) and a small peak for cannabinol at 311.2 (abundance 21.292) and HU-336 at 329.21 (abundance 8.195). Other cannabinoids identified by the library were cannabidiol at 315.23 (abundance 100) and HU-331 at 329.21(abundance 8.195). Moreover, DART analysis of the dried stalks of *Cannabis* sativa plant showed the unique peak of THC at M+ 315.16 (Figure 5). DART analysis of a sample of caffeine showed the peak of caffeine at M+ 195.08 (abundance 39.034) (Figure 6).

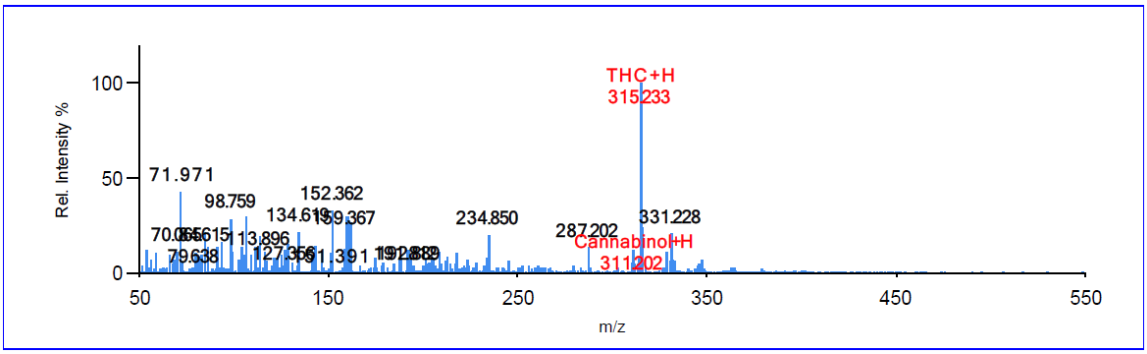

Figure 3 DART+ chart showing the presence of THC peak at M+ 315.233 ([M+H]⁺) in *Cannabis sativa* resin

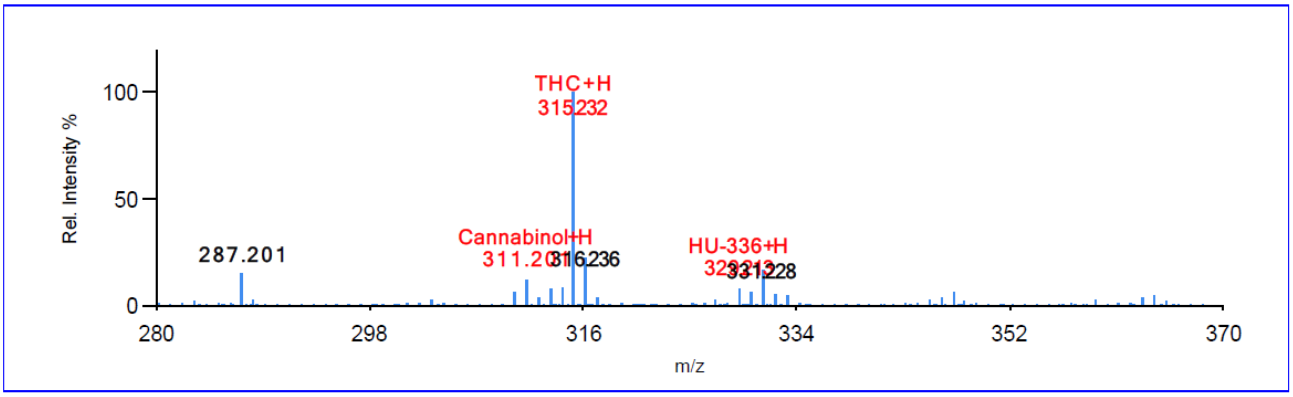

Figure 4 DART+ spectra $[M+H]^+$ showing the presence of THC peak at M+ 315.233 in *Cannabis sativa* resin

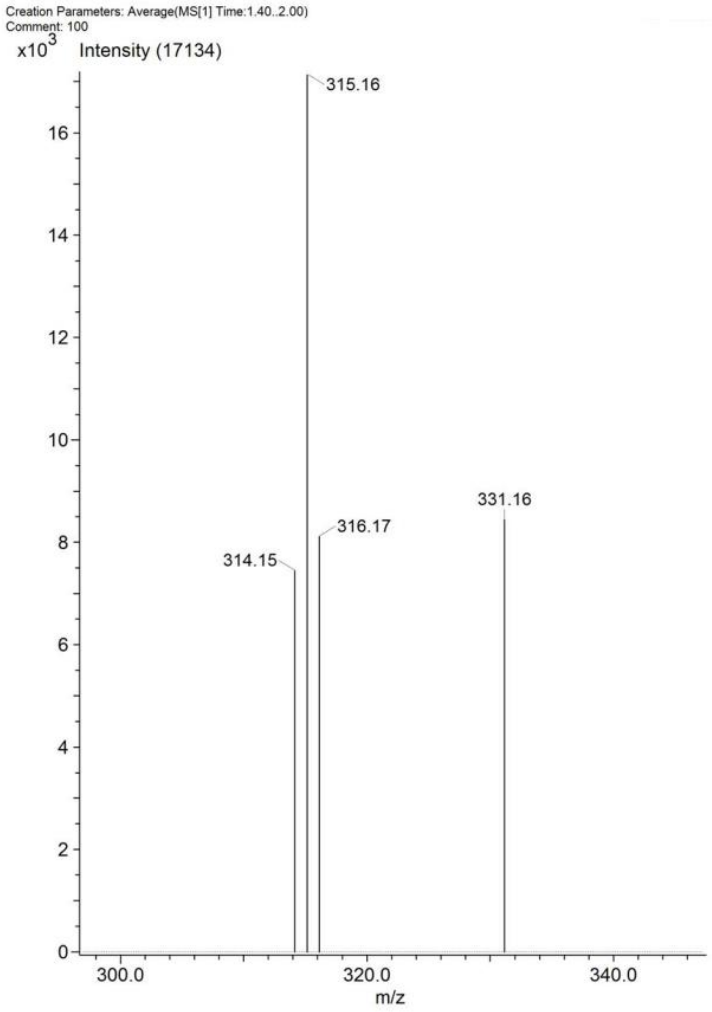

Figure 5 DART+ mass spectra $[M+H]^+$ showing the unique peak of THC at M+ 315.16 in dried stalks of *Cannabis sativa*

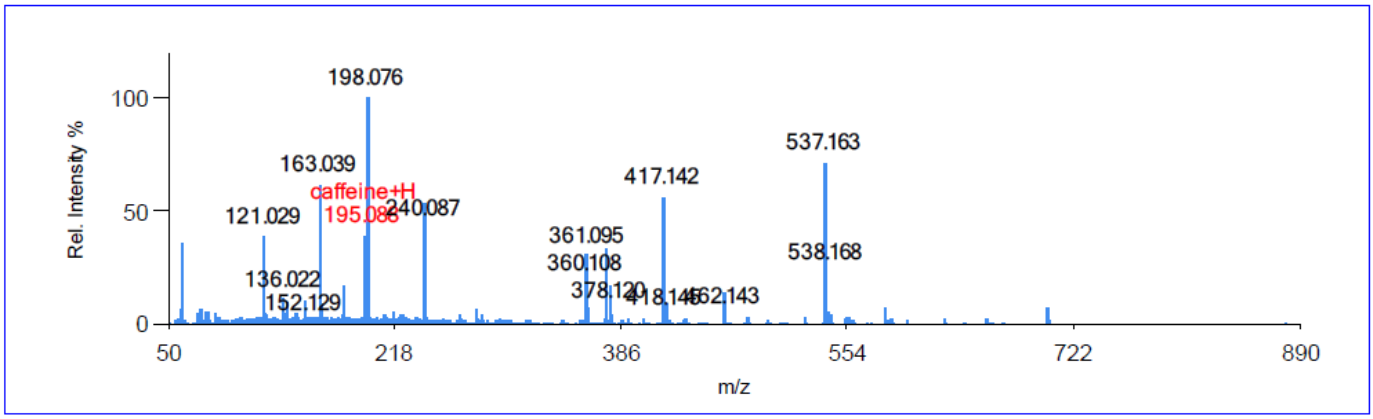

Figure 6 DART+ mass spectra $[M+H]^+$ showing the peak of caffeine at M+ 195.08 in a sample of caffeine

4. DISCUSSION

In the present study, the new mass analysis tool "direct analysis in real time" (DART) equipped with NIST-14 library was used for the first time in the detection of THC in seized marijuana and hashish (resin) samples in Egypt. Our results showed that DART displayed a single high peak for THC in cannabis leaves and resin as well as the dried stalks of the cannabis plant. The cannabis products marijuana and hashish are by far the most abused drugs globally. The *Cannabis sativa* plant chemistry is complex with more than 483 compounds including the C21 terpenophenolic compounds known as cannabinoids which are unique to the plant (El-Sohly et al., 2017). Delta-9-tetrahydrocannabinol (THC) is the most abundant cannabinoid in *Cannabis sativa* plant and is found in the plant's flowering, leaves, stalks, roots and resin (Ashton, 2001). It is also the major psychoactive constituent responsible for the mood altering and other effects of cannabis on the central nervous system perceived when smoking herbal cannabis or hashish (Huestis, 2002). The potency of a cannabis product is described by its THC concentration and has been increasing steadily over the past years through selective cultivation from 3% to 12-16% or even higher (Potter et al., 2008; Swift et al., 2013; Souleman et al., 2017).

Cannabinoids are produced in the plant in the form of their carboxylic acids which upon exposed to heat, light or after prolonged storage are decarboxylated to their corresponding neutral cannabinoids. Therefore, in the fresh plant, THC is present in its acidic form tetrahydrocannabinolic acid (THCA) and is converted by heat e.g., smoking the plant material to its psychoactive form THC (Brenneisen, 2006). Consequently, several analytical methods have been developed that aim mainly to detect the presence of THC in seized samples of cannabis (Hazekamp et al., 2016; Pacifici et al., 2017; Gaafar et al., 2018). In the present study, the DART+ spectra [M+H]+ show high THC peak at M+ 315.233 in both the leaves and resin of cannabis. The results indicated that the protonated THC [M+H+] was the most prominent peak in the DART mass spectra.

Cannabidiol is another common cannabinoid which is a non-psychoactive and that gained wide interest because of its pharmacological properties such as neuroprotection (Iuvone et al., 2009). The presence of cannabidiol in the samples was detected by the DART library NIST14. Other minor cannabinoids are cannabichromene, cannabigerol and cannabidivarin which are non-psychoactive were not detected. Cannabinol, which is approximately 10% as psychoactive as THC (Huestis, 2002) is essentially an oxidative degradation product of THC and its relative abundance increases as samples age (Thomas and El-Sohly, 2015). In the present study, DART+ spectra [M+H]+ showed the presence of small peak for cannabinol at M+ 311.2 in *Cannabis sativa* resin.

Interestingly, DART+ analysis with NIST14 library allowed the detection of the synthetic cannabinoids such as HU-336 and HU-331 at M+ 329.21 and JWH-175 at M+ 328.20. HU-331, HU-336, HU-345 are cannabinoic quinines. The cannabidiol hydroxyquinone, also known as HU-331, is an oxidation product of cannabidiol (Peters and Kogan, 2007), the major non-psychotropic cannabinoid in the *Cannabis sativa* plant. HU-331 has been suggested as a short-lived human oxidative metabolite of cannabidiol (Ujváry and Hanuš, 2016). Very small peaks for HU-336 were observed in the DART+ spectra which could possibly represent an oxidation produced for cannabidiol in the plant. Contrarily, JWH-175 (3-(1-Naphthalenylmethyl)-1-pentyl-1H-indole) is a synthetic cannabinoid which is illegally marketed for its psychoactive cannabis-like effects (Roque-Bravo et al., 2023). JWH-175 was not observed in the DART spectra but identified by the NIST14 library in minute amount.

The THC content varies between the different parts of the cannabis plant being most abundant in the flowers (10-12%), leaves (1-2%) and least in the stalks (0.1-0.3%) and roots (< 0.03%) (United Nations, 2009). Our DART analysis of the dried stalks of cannabis plant showed the presence of peak of THC at M+ 315.16. This result indicated the ability of DART+ to detect minute concentrations of THC in the suspected samples.

5. CONCLUSION

The DART+ analysis presented here allows the rapid and accurate detection of THC, the major psychoactive constituent in herbal cannabis from the different parts of the plant; leaves and dried stalks and the resin. The technique is easy to use and showed high sensitivity, being able to detect the presence of small amounts of TCH in the dried stalks of the *Cannabis sativa* plant. DART+ can be used for the routine analysis of suspected samples.

Author contribution

OMEAS and SS conducted the research and analysis, wrote and prepared the manuscript.

Acknowledgement

The authors acknowledge the late Professor Alaa El-Din M. Gaafar, Department of Photochemistry, National Research Centre, Cairo, Egypt for his help in the DART analysis.

Informed consent

Not applicable.

Ethical approval

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Abdel-Salam OME, Galal AF, Elshebiney SA, Gaafar AEDM. International Aspects of Cannabis Use and Misuse: Egypt. In: Preedy V (editor), Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis and Treatment, 1st Edition. Academic Press, Elsevier Science Publishing Co Inc 2017; 505-516.
2. Abdel-Salam OME. The harm of cannabis in adolescents. Biomed Pharmacol J 2019; 12(2):495-498.
3. Ashton CH. Pharmacology and effects of cannabis: A brief review. Br J Psychiatry 2001; 178:101-106.
4. Brenneisen R. Chemistry and analysis of phytocannabinoids and other cannabis constituents. In: El-Sohly MA (editor). Forensic Science and Medicine: Marijuana and the Cannabinoids. Totowa NJ: Humana Press Inc 2006; 17-49.
5. Brown H, Oktem B, Windom A, Doroshenko V, Evans-Nguyen K. Direct Analysis in Real Time (DART) and a portable mass spectrometer for rapid identification of common and designer drugs on-site. Forensic Chem 2016; 1:6 6-73.
6. Chernetsova ES, Morlock GE, Revelsky IA. DART mass spectrometry and its applications in chemical analysis. Russ Chem Rev 2011; 80(3):235-255.
7. Dahl RE. Adolescent brain development: A period of vulnerabilities and opportunities. Keynote address. Ann N Y Acad Sci 2004; 1021:1–22.
8. El-Shohly MA. Chemical constituents of cannabis. In: Grotenhermen F, Russo E (editors), Cannabis and Cannabinoids. Pharmacology, Toxicology and Therapeutic Potential. New York: Haworth Press Inc 2002; 27-36.
9. El-Sohly MA, Radwan MM, Gul W, Chandra S, Galal A. Phytochemistry of *Cannabis sativa* L. In: Kinghorn AD, Falk H, Gibbons S, Kobayashi J (editors). Phytocannabinoids Unraveling the Complex Chemistry and Pharmacology of Cannabis sativa. Progress in the Chemistry of Organic Natural Products. Switzerland, Springer International Publishing 2017; 1-36.
10. Gaafar AEDM, Abdel-Salam OME, El-Shebiney SA, Ezzat HAM. 1H-NMR study of unheated and heated marijuana and hashish extracts from cannabis seizures in Egypt. Chem J 2018; 1(2):193-199.

11. Gambaro V, Dell-Acqua L, Farè F, Froldi R, Saligari E, Tassoni G. Determination of active constituents in Cannabis preparations by high-resolution gas chromatography/flame ionization detection and high-performance liquid chromatography/UV detection. Anal Chim Acta 2002; 468:245–254.
12. Hazekamp A, Tejkalova K, Papadimitriou S. Cannabis: From cultivar to chemovar II—A Metabolomics. Approach to cannabis classification. Cannabis Cannabinoid Res 2016; 1(1): 202-2015.
13. Huestis MA. Cannabis (Marijuana)—Effects on Human Behavior and Performance Cannabis (Marijuana)—Effects on Human Behavior and Performance. Forensic Sci Rev 2002; 14: 15.
14. Ilias Y, Rudaz S, Mathieu P, Christen P, Veuthey JL. Extraction and analysis of different *Cannabis* samples by headspace solid-phase micro extraction combined with gas chromatography-mass spectrometry. J Sep Sci 2005; 28:2293–2300.
15. Iuvone T, Esposito G, De-Filippis D, Scuderi C, Steardo L. Cannabidiol: A promising drug for neurodegenerative disorders? CNS Neurosci Ther 2009; 15(1):65-75.
16. Kawamura M, Kikura-Hanajiri R, Goda Y. Simple and Rapid Screening for Methamphetamine and 3, 4-Methylenedioxy methamphetamine (MDMA) and Their Metabolites in Urine Using Direct Analysis in Real Time (DART)-TOFMS. Yakugaku Zasshi 2011; 131(5):827–833.
17. Mechoulam R, Gaoni Y. The absolute configuration of delta-1-tetrahydrocannabinol, the major active constituent of hashish. Tetrahedron Lett 1967; 12:1109-11.
18. Musah RA, Domin MA, Walling MA, Shepard JRE. Rapid identification of synthetic cannabinoids in herbal samples via direct analysis in real time mass spectrometry. Rapid Commun Mass Spectrom 2012; 26(9):1109–1114.
19. Pacifici R, Marchei E, Salvatore F, Guandalini L, Busardò FP, Pichini S. Evaluation of cannabinoids concentration and stability in standardized preparations of cannabis tea and cannabis oil by ultra-high performance liquid chromatography tandem mass spectrometry. Clin Chem Lab Med 2017; 55(10):1555-1563.
20. Peters M, Kogan NM. HU-331: A cannabinoid quinone, with uncommon cytotoxic properties and low toxicity. Expert Opin Investig Drugs 2007; 16(9):1405-13.
21. Potter DJ, Clark P, Brown MB. Potency of Δ^9–THC and other cannabinoids in cannabis in England in 2005: Implications for psychoactivity and pharmacology. J Forensic Sci 2008; 53:90-94.
22. Roque-Bravo R, Silva RS, Malheiro RF, Carmo H, Carvalho F, Da-Silva DD, Silva JP. Synthetic cannabinoids: A pharmacological and toxicological overview. Annu Rev Pharmacol Toxicol 2023; 63:187–209.
23. Souleman AMA, Gaafar AE-DM, Abdel-Salam OM, El-Shebiney SA. Determination of delta-9-tetrahydrocannabinol content of cannabis seizures in Egypt. Asian Pac J Trop Med 2017; 10:311-314.
24. Stolker AAM, Schoonhoven J, Vries AJ, Bobeldijk-Pastorova I, Vaes WHJ, Berg RV. Determination of cannabinoids in cannabis products using liquid chromatography- ion trap mass spectrometry. J Chromatogr A 2004; 1058(1):143–151.
25. Swift W, Wong A, Li KM, Arnold JC, Mc-Gregor IS. Analysis of cannabis seizures in NSW, Australia: Cannabis potency and cannabinoid profile. PLoS One 2013; 8:e70052.
26. Thomas BF, El-Sohly MA. The Analytical Chemistry of Cannabis: Quality Assessment, Assurance and Regulation of Medicinal Marijuana and Cannabinoid Preparations. 1st Edition, Elsevier, Amsterdam 2015.
27. Ujváry I, Hanuš L. Human metabolites of cannabidiol: A review on their formation, biological activity and relevance in therapy. Cannabis Cannabinoid Res 2016; 1(1):90-101.
28. United Nations. Recommended methods for the identification and analysis of cannabis and cannabis products, Laboratory and Scientific Section, United Nations Office on Drugs and Crime, Vienna. Sales No. E.09.XI.15. United Nations publication 2009.
29. World Drug Report. Analysis of drug markets. Opiates, cocaine, cannabis, synthetic drugs. United Nations Office for Drug and Crime (UNODC). Sales No. E. 18.XI.9. United Nations publication 2018.
30. World Drug Report. United Nations Office for Drug and Crime (UNODC). Sales No. E.20.XI.6. United Nations publication 2020.